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Stable Isotope Labeling, *in Vivo*, of D- and L-Tryptophan Pools in *Lemna gibba* and the Low Incorporation of Label into Indole-3-Acetic Acid[1]

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ABSTRACT

We present evidence that the role of tryptophan and other potential intermediates in the pathways that could lead to indole derivatives needs to be reexamined. Two lines of *Lemna gibba* were tested for uptake of [^{15}N-*indole*]-labeled tryptophan isomers and incorporation of that label into free indole-3-acetic acid (IAA). Both lines required levels of L-[^{15}N]tryptophan 2 to 3 orders of magnitude over endogenous levels in order to obtain measurable incorporation of label into IAA. Labeled L-tryptophan was extractable from plant tissue after feeding and showed no measurable isomerization into D-tryptophan. D-[^{15}N]tryptophan supplied to *Lemna* at rates of approximately 400 times excess of endogenous D-tryptophan levels (to yield an isotopic enrichment equal to that which allowed detection of the incorporation of L-tryptophan into IAA), did not result in measurable incorporation of label into free IAA. These results demonstrate that L-tryptophan is a more direct precursor to IAA than the D isomer and suggest (a) that the availability of tryptophan *in vivo* is not a limiting factor in the biosynthesis of IAA, thus implying that other regulatory mechanisms are in operation and (b) that L-tryptophan also may not be a primary precursor to IAA in plants.

Many studies of IAA biosynthesis have shown that TRP[4] is the primary precursor of IAA by one of several different pathways (for reviews, see refs. 1, 24). However, studies of *in vivo* biosynthesis of IAA in maize seedlings have demonstrated that conversion of TRP to IAA does not occur at significant rates (9, 17, 21). The simple model for TRP involvement in IAA biosynthesis is further complicated by the natural occurrence of the 12 carbon indole, IBA, in some plant tissues (8, 23). The metabolic conversion of an indolic precursor to IBA has not been demonstrated; however, the side chain reduction of IBA to IAA has been shown to occur in plants (10, 12). These results present an interesting problem with respect to the hypothesis that TRP is the primary precursor to indolealkanoic acids in plants. The *in vivo* role of TRP and other potential intermediates in pathways that could lead to indole derivatives clearly needs to be reexamined.

Recently, D-TRP, rather than the much more abundant L isomer, has been proposed to be a direct biosynthetic precursor of IAA based on evidence of gibberellin enhanced racemization of L-TRP to D-TRP, which could then be transaminated to indolepyruvic acid (19, 20). The evidence for the *in vivo* operation of this pathway is difficult to interpret because correction of the specific activity of the applied labeled compounds, necessitated by the differences in uptake and size of internal pools, was not done.

Our studies of IAA biosynthesis *in vivo* have been facilitated by the use of an IAA overproducing mutant, *Lemna gibba* jsR_1 (26). The mutant along with the parent *L. gibba* G-3 line form a unique system for the sterile feeding of compounds to intact plants without wounding effects (6, 26, 27).

In this report we describe the feeding of specific stable isotope labeled compounds to *Lemna* coupled with analysis by mass spectrometry. The incorporation of [^{15}N-*indole*]TRP into endogenous pools of either D- or L-TRP, and the metabolism of labeled TRP into either the isomeric TRP or into free IAA was measured. The metabolic path of the labeled indole nitrogen was analyzed by comparing the increase in ions of mass 131, the major ion fragment of both TRP and IAA when ^{15}N was present in the indole ring, over the natural abundance of ion fragments of mass 131 in extracted free IAA. Using this method, the levels of utilization of applied TRP isomers for IAA biosynthesis were measured. Only low levels of *in vivo* incorporation of ^{15}N from L-TRP into free IAA were found even when the levels of L-TRP in the plant were up to 1600 times the normal endogenous pool size. No measurable incorporation of ^{15}N from D-[^{15}N]TRP into free IAA was obtained. D-[^{15}N]TRP was fed at a level so as to yield an *in vivo* isotopic enrichment equal to that obtained in experiments using L-TRP. The current study is preliminary to a much larger and detailed use of stable isotopes for measurement of indole metabolism in plants now being conducted in our laboratory.

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[4] Abbreviations: TRP, tryptophan; IBA, indole-3-butyric acid; PL, parental line; GC-MS-SIM, selected ion monitoring GC-MS; DIP-MS, direct insertion probe-MS; EI, electron impact.

MATERIALS AND METHODS

Plant Material

Sterile cultures of *Lemna gibba* G-3 (PL) were maintained on E medium at 26 to 28°C under growth conditions described previously (26). Two liter flasks containing 1 L of medium were maintained in 8 h light, 16 dark for 45 d before subculturing. *L. gibba* G-3 jsR_1, a spontaneous regenerant from tissue culture (26), was maintained under identical growth conditions as PL. Plants used for feeding experiments were from 30 d cultures; prior studies showed (26) that an increase in the level of IAA in jsR_1 begins at this time. For fresh weight determinations, either before or after feedings, plants were collected on a nylon mesh, washed with distilled water, and dried with paper towels to remove surface water. Plants were frozen in liquid N_2 and stored at −80°C prior to analyses.

Instrumentation

Chiral separation of underivatized TRP isomers was done by HPLC using a Waters[5] 600 MS solvent pumping system with a Chiralpak-WH HPLC column (4.6 × 50 mm: Diacel, Tokyo) maintained in a column heater at 50°C. Solvent was 0.25 mM copper sulfate (aqueous) at 1.5 mL/min (HPLC system 1). Compounds eluting from the column were detected using a Waters 450 variable wavelength UV detector at 282 nm, a Shimadzu RF535 fluorescence detector set at 280 nm excitation, 360 nm emission, and/or a Beckman 171 radioactivity detector with 125 μL solid scintillant flow cell. Complete separation of D- from L-TRP (retention times 4 and 14 min, respectively) was obtained and either isomer could be detected by fluorescence to a detection limit of less than 5 ng. Extracted TRP was purified using a Waters 6000 dual pump system with two different HPLC columns: an Ultrasphere ODS C18 column (4.6 × 250 mm, Beckman) with a solvent of 25% methanol: 75% water containing 5% acetic acid at 1 mL/min, or a Microsorb C18 5 μ column (4.6 × 120 mm, Rainin) with a solvent of 64% methanol: 36% water containing 2% acetic acid at 1 mL/min (HPLC system 2). HPLC system 3, for purification of IAA, was as previously described (4). MS data were obtained on a Kratos MS25RFA mass spectrometer equipped with a Carlo Erba 5300 GC, Kratos DS90 v. 4.0 software, and a Data General Desktop 30 minicomputer. DIP-MS was done using EI ionization at a source temperature of 250 C. GC-MS-SIM was done using a 15 m 0.32 mm i.d. DB1701 capillary column (J & W Scientific) eluting directly into the source. Other conditions for GC-MS-SIM were essentially as described elsewhere (5).

Separation of Labeled TRP into D and L Isomers

D[^{14}C]- and L-[^{14}C]TRP standards were prepared by resolving D,L-[^{14}C]TRP (57 mCi/mmol; Amersham) using HPLC system 1. Copper was removed by passing the separated isomer fractions through a 5 mL bed volume column of Chelex 100 (Bio-Rad), followed by a distilled water wash. The eluent was reduced *in vacuo*. When retested on Chiralpak-WH, the separated isomer fractions were found to have no measurable contamination by the other isomer and approximately 95% of the total radioactivity was in the one TRP isomer peak.

D,L-[^{15}N-*indole*]TRP (99 atom %; Cambridge Isotopes) was separated into isomer fractions without derivatization using TLC (Chiralplate, Macherey-Nagel, Duren) developed with methanol:water:acetonitrile (5:5:20). TLC was used, rather than HPLC, to avoid potential problems from the copper in the HPLC solvent, which may not be completely removed by the Chelex 100 treatment. Copper can have profound effects on *Lemna* growth even at trace levels. D-[^{15}N]TRP (R_F 0.50) chromatographed below L-[^{15}N]TRP (R_F 0.60) as visualized under UV or by color development following treatment with Ehmann's reagent (7). TRP zones were scraped off the plate, eluted with 2 N ammonium hydroxide, concentrated, resuspended in 50% methanol, and purified using HPLC system 2 (Ultrasphere). The TRP peak was streaked on a second TLC plate and eluted as above. The resulting solution was shown to be 92% D-, 8% L-TRP based on peak area using HPLC system 1. DIP-MS of the D-[^{15}N]TRP gave a spectrum characteristic of [^{15}N-*indole*]TRP (m/z 205 M^+ and base peak m/z 131). L-[^{15}N]TRP (99 atom %) was available commercially (KOR Isotopes) and was shown by HPLC system 1 to have no detectable D-[^{15}N]TRP.

Endogenous TRP Extractions from *Lemna*

TRP was extracted from *Lemna* (PL and jsR_1) by previously published methods with only slight variation, and then quantified by isotope dilution analysis (15, 16). Plant material, 1 or 2 g for L-TRP extractions or at least 20 g for D-TRP extractions, was frozen in liquid nitrogen and homogenized in 70% acetone (5 mL/g of tissue). The corresponding radioactive labeled isomer of TRP (57 mCi/mmol; 40,000 dpm) was added and the extract allowed to equilibrate for several hours at 4°C. The extract was centrifuged (2500*g*, 5 min; Sorvall GLC with HL-4 rotor) and the pellet was resuspended in 70% acetone and centrifuged again (two times). The combined supernatants were filtered (Whatman No. 1), reduced to a water phase *in vacuo*, diluted three to four times with distilled water, and applied to a column of acidified Dowex 50W-X8 (20–50 mesh, 15 mL bed volume). The column was washed with three bed volumes of distilled water and TRP eluted with three to four bed volumes of 2 N ammonium hydroxide. The eluted fractions containing radioactivity were reduced to near dryness *in vacuo*, resuspended in 50% methanol and precipitate removed by centrifugation at 2000*g* (Tomy HF-120 Capsule centrifuge using 1.5 mL plastic tubes). The supernatant was fractionated using HPLC system 2. TRP eluted as a single peak at R_t 10 min. Radioactive labeled fractions were pooled and reduced in volume for determination of TRP isomer ratios or quantification of TRP levels. TRP extractions were separated into D and L isomer fractions using HPLC system 1. The ratio of the level of the two isomers was determined by both the ratio of peak areas and direct isotope dilution quantification of isomer levels. For quantification of each isomer via chiral HPLC, peak areas and percent

[5] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply approval to the exclusion of other products or vendors that may be suitable.

recovery of label were used for isotope dilution analysis (15). For quantification on the C18 Microsorb column (HPLC system 2), fractions of each isomer from chiral HPLC were washed from the Chelex 100 column, reduced in volume and analyzed separately (TRP peaks eluting at 5 min) with data also evaluated by isotope dilution analysis (15). For determination of total TRP extracted, the samples obtained from the C18 Beckman column were injected directly on the C18 Microsorb column and quantified as described above.

Labeling Endogenous TRP Pools with ^{15}N

Labeling of the endogenous TRP pools in PL or jsR_1 with either D- or L-[^{15}N]TRP was accomplished using 50 mL conditioned E medium (transferred under sterile conditions from flasks at d 30 of the culture cycle). The medium was added to large Petri dishes (15 cm diameter) that contained either the stable isotope label or an equal amount of unlabeled L-TRP (Pfaltz and Bauer, Waterbury, CT) or to control dishes with nothing added. Labeled or unlabeled L-TRP (400–800 μM) was used in these experiments, and an estimated 8 g fresh weight of plants were added. The plants were weighed at the end of the experiment, and this value was used to calculate the milligrams TRP/g fresh weight used. For L-TRP this was 0.4 to 1.6 mg/g fresh weight. The levels of labeled D-TRP used were 3 to 4.4 μM or 5 to 6 μg/g fresh weight. Thus, the levels of D-TRP used were approximately 1.3% of that provided in the L-TRP incubations. Plants were added to media until the surface of the medium was completely covered (5–10 g fresh weight of PL or jsR_1). The dishes were sealed with Parafilm and returned to the growth chamber (conditions as described for stock cultures above) for up to five days. Uptake of exogenous L-TRP was followed by direct analysis of the media samples (HPLC system 2) to determine TRP depletion from the medium. Because of the lower levels of D-TRP utilized, its uptake was determined in a parallel experiment using unlabeled D-TRP with D-[^{14}C]TRP added as a tracer.

Increases in extractable TRP pool sizes were quantified by bulk extraction of TRP from plant material, as previously described for endogenous TRP pool determinations. ^{15}N incorporation into the extractable L-TRP pool was verified by DIP-MS analysis of total TRP extracted from plants fed L-[^{15}N]TRP. Racemization of fed L-[^{15}N]TRP to the D isomer was determined by chiral HPLC of TRP extracts. Levels of D-TRP following incubation of plants with L-TRP were measured by peak areas from HPLC. To analyze the L-TRP fraction for possible racemization after feeding of D-[^{15}N]TRP, Chiralplate TLC isolation of L-TRP was done, as previously described. DIP-MS of the purified D- or L-TRP fraction allowed detection of even low levels of incorporation of ^{15}N as measured by an increase in m/z 131 in the extracted TRP isomers.

Free IAA Analyses

Incorporation of ^{15}N into endogenous IAA in *Lemna* PL and jsR_1 was analyzed by extraction of free-IAA from plant tissue by previously published methods (4) with slight variation. Following feeding, plant tissue (4–8 g fresh weight) was frozen in liquid nitrogen and homogenized in extraction buffer (65% isopropanol, 35% 0.2 M imidazole, pH 7; 4 mL/g) containing [$^{13}C_6$]IAA, as an internal standard (5), and [^{3}H]IAA, as a tracer. The supernatant from the homogenate was collected after equilibration with the standard for several hours at 4°C, the pellet extracted again in buffer (×2), and the combined supernatants reduced *in vacuo* to the water phase. The water phase was diluted to 75 mL and placed on a DEAE-Sephacel-acetate (Pharmacia) mini-column (bed volume 15 ml). Washing and eluting of IAA were as previously described (8). HPLC purification of IAA containing fractions was carried out using HPLC system 3 and the IAA containing fraction was prepared for GC-MS-SIM analysis by methylation with diazomethane (5). Measurement of incorporation of the ^{15}N label from fed TRP isomers into the free IAA pool was made by comparison of the 130/131 peak ratios in control plants and those fed [^{15}N]TRP for up to 5 days.

RESULTS

Data from extractions of endogenous D- and L-TRP from jsR_1 and PL harvested from 30 d cultures are summarized in Table I. TRP analysis using C18 HPLC (HPLC system 2 using the Microsorb column) showed that in jsR_1 the amount of D- and L-TRP was 0.007 and 1.43 μg/g fresh weight, respectively, and in PL the amount of D- and L-TRP was 0.003 and 0.96 μg/g fresh weight, respectively. The determination of D- and L-TRP in jsR_1 and PL by chiral HPLC and isotope dilution analysis gave similar values. The endogenous D-TRP, calculated from these data, was 0.3 to 0.5% of the total TRP level. Similarly, the percentage of D-TRP determined directly by chiral HPLC and peak analysis yielded values of 0.6 ± 0.3% from three separate extractions. Therefore, the levels of exogenous L-[^{15}N]TRP fed to the plant were 400 to 1600 times the endogenous L-TRP in both lines of *Lemna*. These levels of TRP caused no visible signs of toxicity to the plants. D-[^{15}N]TRP feedings were done at approximately 800 times the endogenous D-TRP levels.

For both PL and jsR_1, the loss of TRP from the media (Fig. 1) and the increase in extractable free TRP measured in the plants after 120 h incubation showed that greater than 98% of the label was taken up and retained as free L-TRP by the plants. This uptake occurred mostly during the first 60 h of incubation; thus, high levels of L-TRP were available within the plant during most of the incubation time for use in IAA synthesis, protein biosynthesis, and other metabolism. L-[^{15}N]TRP extracted from the plant tissue and analyzed by DIP-MS following 120 h incubation showed no measurable isotopic dilution. In four separate experiments, racemization of fed L-[^{15}N]TRP to the D-TRP pool was not seen over a 120 h

Table I. *Endogenous Levels of Tryptophan Isomers from the PL and jsR_1 Lines of L. gibba at the Beginning of the Incubation Period*

Isomer	jsR_1	PL
	ng/g fresh weight[a]	
L-Tryptophan	1430 ± 290 (3)	960 ± 280 (2)
D-Tryptophan	7 ± 5 (3)	3 ± 2 (3)

[a] Mean ± standard or mean deviation (*n*).

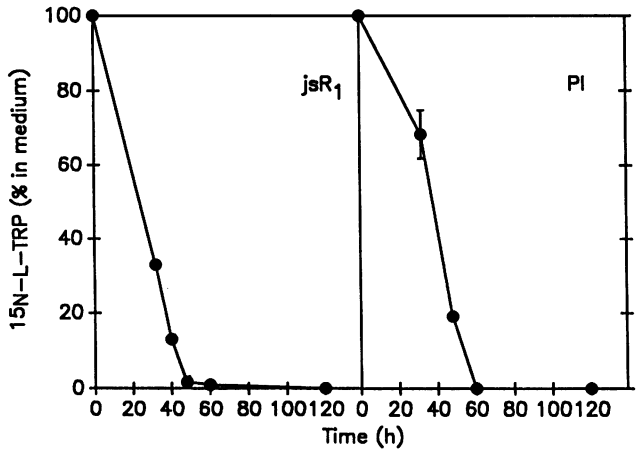

Figure 1. Concentration of L-[^{15}N]TRP (400 μM) in the incubation medium measured by HPLC analysis. Time points to determine uptake into jsR$_1$ at 32, 40, and 60 h were from a different set of experiments using unlabeled 400 μM L-TRP; all other analyses were done with the same medium used for determination of ^{15}N incorporation into IAA. Data are the average from three separate incubation dishes (biological replicates) and error bars indicate standard deviation (n = 3) larger than the marked point.

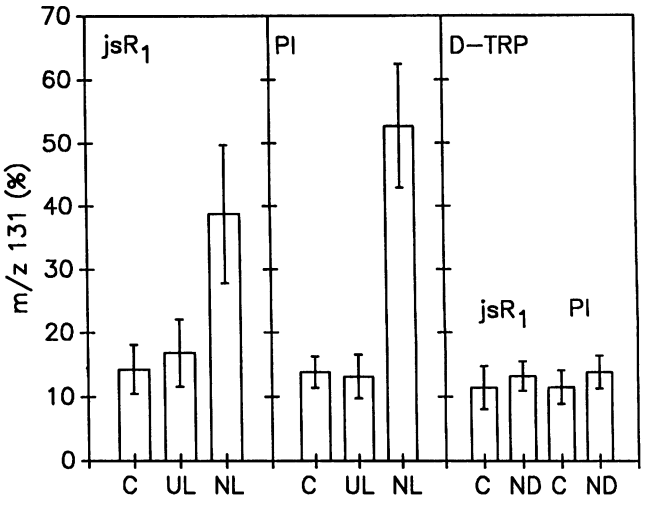

Figure 3. Percentage of m/z 131 (relative to m/z 130 = 100%) mass fragment ion (the ratio between the unlabeled quinolinium ion and the quinolinium ion with one stable isotope incorporated) in free IAA extracted from *L. gibba* after feeding for 5 days with: no added TRP (C); unlabeled L-TRP (UL); L-[^{15}N]TRP (NL); D-[^{15}N]TRP (ND). Error bars indicate standard deviation (n = 3–6 independent biological replicates).

incubation. This was confirmed by chiral HPLC quantification, which showed no increase in the D-TRP pool following high level incubation with L-TRP. Uptake studies of D-[^{14}C]TRP showed that about 80% was removed from the medium by the end of 60 h of incubation (Fig. 2). Extraction and chiral HPLC analysis of D-TRP following D-[^{15}N]TRP feeding showed an increase in endogenous D-TRP pools of 210 times the normal levels. Racemization of this D-[^{15}N]TRP into the L-TRP pool does not appear to occur since no increase over the natural abundance of ion mass 131 in L-TRP was detected by DIP-MS.

[^{15}N]Indole from L-[^{15}N]TRP is incorporated into free IAA (Fig. 3), as seen by the increase in the abundance of ion mass 131, which is 2.5 to 3 times that of controls for both jsR$_1$ and PL in the L-TRP feedings. As shown in Figure 3, the normal abundance of the m/z 131 ion in TRP is about 12% of the m/z 130 abundance (due primarily to the natural occurrence of ^{13}C). Thus the increase in the m/z 131 ion noted in the isolated IAA from the L-[^{15}N]TRP-fed plants represents an isotopic enrichment of 20 to 30% of the free IAA in these tissues. Based on the level of endogenous free IAA determined for these tissues at this stage of growth cycle (12–26 ng/g fresh weight), 4 to 7 ng/g of IAA was labeled. Thus, less than 2 ppm of the labeled L-TRP fed was actually incorporated into free IAA measured in both lines of *Lemna*, even though L-[^{15}N]TRP accounted for 98% of the total free TRP pool. Probably due to the extremely high level of applied L-TRP, no measurable change in internal level occurred after the initial uptake period until the end of the 120 h incubation. Since the plants did grow during the incubation period, some incorporation into protein and other metabolic uses must have taken place; however, such processes did not consume measurable amounts of L-TRP relative to the levels provided. Plants fed labeled D-TRP showed no significant increases in ion mass 131 in free IAA extracted (Figure 3), even though the D-TRP pool was essentially totally labeled. For both TRP isomers and in both plant lines, no significant changes in free IAA levels were measured following feeding experiments.

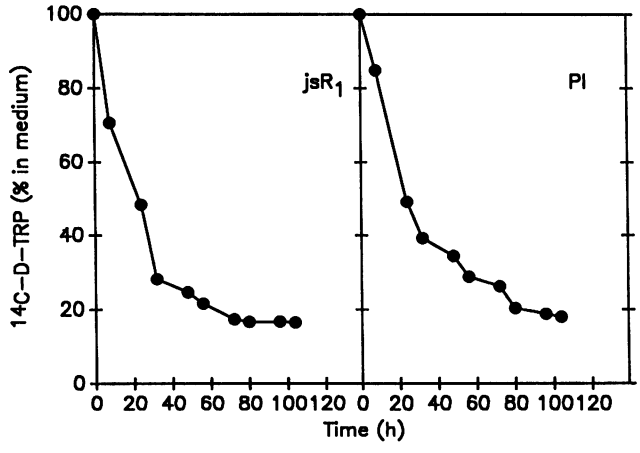

Figure 2. Concentration of D-[^{14}C]TRP in medium containing 4.4 μM D-TRP and tracer levels of D-[^{14}C]TRP from PL and jsR$_1$ lines of *L. gibba*. Data are the average of values obtained from duplicate incubations.

DISCUSSION

Several previous reports on the levels of free TRP in plant material have given a range of values from 1 to 500 μM (9, 16, 28, 29), with the highest values found in seed material. Growing vegetative parts of the plant usually contain between 1 and 10 μM free TRP (9, 14), and such levels are consistent

with our current finding of between 5 and 7 μM free TRP in *Lemna*.

Our results show that the conversion of applied labeled TRP into free IAA in *Lemna* occurs at rates significantly lower than would be expected based on the assumption that TRP is a primary precursor of IAA. These low rates are seen even when endogenous TRP levels are increased by three orders of magnitude following feeding TRP to the plants. *Lemna* is a useful plant for these studies in that it will take up the L isomer essentially quantitatively and will also accumulate significant quantities of D-TRP from the medium.

Low rates of metabolism of TRP to IAA (11), or lack of measurable metabolism of TRP to IAA (30) have been reported for other plant systems. Only in tissue segments or cell free studies of IAA biosynthesis has it been possible to demonstrate growth in response to TRP or significant conversion of TRP to IAA during short-term incubations (18, 19, 25). In our initial experiments, measurable incorporation of ^{15}N label into IAA was not observed when L-TRP was supplied at levels of 200 μg/g fresh weight for 2 d. The concentration of L-TRP (400 μg/g fresh weight) we found necessary to observe labeling of IAA (Fig. 3), the low incorporation of label into IAA, and the lack of an increase in free IAA levels in plants fed L-TRP suggests that the level of endogenous L-TRP is not an important factor in the regulation of IAA biosynthesis. Moreover, even plants fed L-[^{15}N]TRP at four times the level used in this study (1600 μg/g fresh weight) did not show increased incorporation of ^{15}N label into IAA or increased IAA levels. These results show that endogenous levels of IAA are not regulated by availability of TRP, and show that massive supplementation of endogenous pools is necessary in order for applied label to be utilized in the biosynthesis of IAA.

Based on the assumption that TRP is the major precursor to IAA, an apparent half-life of IAA in PL and jsR_1 was calculated to be 421 and 313 h, respectively (using equations previously described [9 and references cited therein] and the ^{15}N enrichments in IAA shown in Fig. 3):

$$\log (C_0/C_t) = (kt/2.303)$$

where k is the first order rate constant, C_0 is the percentage of unlabeled IAA at the initial sampling (87%, due to the natural heavy isotope enrichment), and C_t is the percentage of unlabeled IAA (66.7 and 71.4% for PL and jsR_1, respectively) after time, t (120 h).
The turnover is then given by:

$$t_{1/2} = (\ln 2/k).$$

An additional assumption, that pools of TRP are uniformly labeled, was made. The calculated turnover rate would not be correct if this assumption, or the assumption that TRP is the major precursor, is false.

In other plants that have been studied, turnover rates for IAA range from minutes to as long as 3 h (9). The turnover rates calculated here for *Lemna* of almost 2 weeks or more are unrealistic, which indicates that TRP is not a major precursor of IAA.

The limited incorporation of TRP into IAA in *Lemna* seen in these experiments can be explained if neither L-TRP nor D-TRP is a direct precursor to IAA, but L-TRP can be metabolized to ultimately yield the precursor. Alternatively, an internal cellular compartment could exist into which the vast bulk of exogenous TRP does not enter and which is the location of the committed step of IAA biosynthesis. Reports that TRP synthesis is compartmentalized (29), and that TRP synthetase is localized in the chloroplast (2, 14) make this a feasible postulate. Also, evidence exists suggesting that some IAA biosynthesis may be localized in plastids (3, 20, 22) or mitochondria (13). Nonetheless, we propose as a working hypothesis that TRP is not the primary precursor for IAA, and there is evidence from other plant systems which further supports this conclusion (L Michalczuk, TJ Cooke, JD Cohen, unpublished results; AD Wright, MG Neuffer, MB Sampson, L Michalczuk, JP Slovin, JD Cohen, unpublished results; P Jensen, RS Bandurski, personal communication).

Although D-TRP fed to *Lemna* may also be unavailable to an internal compartment in which D-TRP is normally metabolized to IAA, such as the chloroplasts (20), the lack of incorporation of labeled D-TRP into IAA at the high concentrations fed to the plants would appear to rule out a role for this isomer in IAA biosynthesis in *Lemna*. D-TRP is probably not a precursor to IAA under any conditions, at least not *in vivo* during periods of normal growth. Specifically, the lack of racemization between L- and D-TRP, the labeling of IAA from L-TRP without changes in the D-TRP pool and the observation that the free IAA level is equal to or greater than the D-TRP level in *Lemna* makes it improbable that D-TRP is an intermediate in IAA biosynthesis.

There were no significant differences between the two lines (PL and jsR_1) in their incorporation of [^{15}N]TRP isomers into IAA (Fig. 3). After 120 h of incubation, virtually all of the L-TRP in the medium had been taken up by the plants of both lines (Figure 1) even though uptake of either D- or L-TRP into jsR_1 was somewhat more rapid than uptake into PL (Figs. 1, 2). The lack of significant differences in TRP metabolism into IAA may indicate that the rate of biosynthesis of IAA is identical in these two lines and that the accumulation of free IAA by jsR_1 (26) is the result of other aspects of IAA metabolism (*i.e.* turnover and conjugation). The experiments described here indicate that the levels of L-TRP present in *Lemna* under normal conditions do not limit the rate of *de novo* biosynthesis of IAA and suggest that the regulated steps in IAA biosynthesis do not involve the conversion of TRP to IAA.

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LITERATURE CITED

1. **Bandurski RS, Nonhebel H** (1984) Auxins. *In* MB Wilkins, ed, Advanced Plant Physiology. Pitman, London, pp 1–20
2. **Berlyn MB, Last RL, Fink GR** (1989) A gene encoding the tryptophan synthase subunit of *Arabidopsis thaliana*. Proc Natl Acad Sci USA **86:** 4604–4608
3. **Brown BH, Crozier A, Sandberg G, Jensen E** (1986) Indole-3-acetic acid and indole-3-ethanol in light grown *Pisum sativum* seedlings and their localization in chloroplast fractions. Phytochemistry **25:** 299–302
4. **Chen K-H, Miller AN, Patterson GW, Cohen JD** (1987) A rapid and simple procedure for purification of indole-3-acetic acid prior to GC-SIM-MS analysis. Plant Physiol **86:** 822–825

5. **Cohen JD, Baldi BG, Slovin JP** (1986) $^{13}C_6$-[benzene ring]-Indole-3-acetic acid: a new internal standard for quantitative mass spectral analysis of indole-3-acetic acid in plants. Plant Physiol **80:** 14–19
6. **Cohen JD, Slovin JP, Bialek K, Chen K-H, Derbyshire MK** (1988) Mass spectrometry, genetics and biochemistry: understanding the metabolism of indole-3-acetic acid. *In* GL Steffens, TS Rumsey, eds, Biomechanisms Regulating Growth and Development (12th Beltsville Symposia in Agricultural Research). Kluwer Academic Publishers, Dordrecht, pp 229–241
7. **Ehmann A** (1977) The Van Urk-Salkowski reagent—a sensitive and specific chromagenic reagent for silica gel thin layer detection and identification of indole derivatives. J Chromatogr **132:** 267–276
8. **Epstein E, Chen K-H, Cohen JD** (1989) Identification of indole-3-butyric acid as an endogenous constituent of maize kernels and leaves. Plant Growth Regul **8:** 215–223
9. **Epstein E, Cohen JD, Bandurski RS** (1980) Concentration and metabolic turnover of indoles in germinating kernels of *Zea mays* L. Plant Physiol **65:** 415–421
10. **Epstein E, Lavee S** (1984) Conversion of indole-3-acetic acid by cuttings of grapevine (*Vitis vinifera*) and olive (*Olea europeae*). Plant Cell Physiol **25:** 697–703
11. **Erdmann N, Schiewer U** (1971) Trypophan-dependent indoleacetic acid biosynthesis from indole, demonstrated by double-labeling experiments. Planta **97:** 135–141
12. **Fawcett CH, Wain RL, Wightman F** (1960) The metabolism of indolylalkenecarboxylic acids and their amides, nitriles and methyl esters in plants. Proc R Soc Lond Ser B **152:** 231–241
13. **Fregau JA, Wightman F** (1983) Natural occurrence and biosynthesis of auxins in chloroplast and mitochondrial fractions from sunflower leaves. Plant Sci Lett **32:** 23–34
14. **Gilchrist DG, Kosuge T** (1980) Aromatic amino acid biosynthesis and its regulation. *In* BJ Miflin, ed, The Biochemistry of Plants, Vol 5. Amino Acids and Derivatives. Academic Press, New York, pp 507–531
15. **Hall PL, Bandurski RS** (1978) Movement of indole-3-acetic acid and tryptophan-derived indole-3-acetic acid from the endosperm to the shoot of *Zea mays* L. Plant Physiol **61:** 425–429
16. **Hanson SD, Cohen JD** (1985) A technique for collection of exudate from pea seedlings. Plant Physiol **78:** 734–738
17. **Jensen P, Bandurski RS** (1989) Attempting to monitor the incorporation of deuterium into indole-3-acetic acid and tryptophan in *Zea mays* grown on deuterium oxide labeled water (abstract No. 566). Plant Physiol **89:** S-95
18. **Kutacek M** (1988) Enzymes of auxin synthesis in plant and tissue cultures. *In* M Kutacek, RS Bandurski, J Krekule, eds, Physiology and Biochemistry of Plants. SPB Academic Publishing, The Hague, pp 57–64
19. **Law D** (1987) Gibberellin-enhanced indole-3-acetic acid biosynthesis: D-tryptophan as the precursor of indole-3-acetic acid. Physiol Plant **70:** 626–632
20. **McQueen-Mason S, Hamilton RH** (1989) The biosynthesis of indole-3-acetic acid from D-tryptophan in Alaska pea plastids. Plant Cell Physiol **30:** 999–1005
21. **Pengelly W, Bandurski RS** (1983) Analysis of indole-3-acetic acid metabolism using deuterium oxide as a tracer. Plant Physiol **73:** 445–449
22. **Sandberg G, Jensen E, Crozier A** (1982) Biosynthesis of indole-3-acetic acid in protoplasts, chloroplasts, and a cytoplasmic fraction from barley (*Hordeum vulgare,* L). Planta **156:** 541–545
23. **Schneider EA, Kazakoff CW, Wightman F** (1985) Gas chromatography-mass spectrometry evidence for several endogenous auxins in pea seedlings organs. Planta **165:** 232–241
24. **Sembdner G, Gross D, Liebisch H-W, Schneider G** (1980) Biosynthesis and metabolism of plant hormones. *In* J MacMillan, ed, Hormonal Regulation of Development. I. Molecular Aspects of Plant Hormones. Springer Verlag, New York, pp 281–454
25. **Sherwin JE, Purves WK** (1969) Tryptophan as an auxin precursor in cucumber seedlings. Plant Physiol **44:** 1303–1309
26. **Slovin JP, Cohen JD** (1988) Levels of indole-3-acetic acid in *Lemna gibba* G-3 and in a large *Lemna* mutant regenerated from tissue culture. Plant Physiol **86:** 522–526
27. **Slovin JP, Tobin E** (1982) Synthesis and turnover of the light-harvesting chlorophyll *a/b*-protein in *Lemna gibba* grown with intermittent red light: possible translational control. Planta **154:** 465–472
28. **Widholm JM** (1971) Control of tryptophan biosynthesis in plant tissue cultures: lack of repression of anthranilate and tryptophan synthetases by tryptophan. Physiol Plant **25:** 75–79
29. **Widholm JM** (1974) Evidence for compartmentation of tryptophan in cultured plant tissues: free tryptophan levels and inhibition of anthanilate synthetase. Physiol Plant **30:** 323–326
30. **Winter** (1966) A hypothetical route for the biogenesis of IAA. Planta **71:** 229–239